



## SERUM INTERLEUKINS (IL-4, IL-10) AND IMMUNOGLOBULIN A AS BIOMARKERS IN PATIENTS WITH GIARDIASIS

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### Abstract

*Giardia lamblia* is the parasite that infects Humans. The parasite is causative agents of giardiasis. It is commonly effect of small intestine and responsible of both acute and chronic diarrheal infection spread worldwide. The present study was aimed to investigates the effect of *G. lamblia* infection on some immunological biomarkers such as, IL-4, IL-10 and immunoglobulin IgA, in patients with giardiasis. During the period of June to August / 2018, one hundred stool samples from patients admitted to the main two hospitals in Babylon province, Iraq, aged (1-50) years were tested for the detection of the parasite by using direct smear. Blood samples recovered from patients were used to measure the level of IL-4, IL-10, and IgA compared to healthy persons (20 sample) by using ELISA test. The results showed infection rate of *G. lamblia* was 34%. According to gender, high infection was noted in males 34.4% more than females (33. 3%). However, this result was not significant. Immunological parameters of (IL-4, IL-10 and IgA) were increased significantly (p <0.05) in infected group compared with control group. The levels of interleukins IL-4 and IL-10 were increase significantly in infected group when compared with control group. The immunoglobulin A concentration was increased significantly in infected group in compared to control group.

**Keywords:** giardiasis, Prevalence rate, interleukins, IL-4, IL-10, Biomarkers.

### Introduction

*G. lamblia* is a pathogenic flagellate that resides in the intestinal tract of humans which attachment to villi of small intestine and caused severe gastrointestinal disease (Al-Sabbawi, 2007; Hill, 1995). This microorganism a worldwide parasite may be leads to chronic diarrhea and mala-bsorption of human (Al-Sabbawi, 2007; Chin, 2000). The symptoms of giardiasis patients mainly suffer from abdominal pain, diarrhea, nausea, chills and fever in addition to weight loss (Ensink *et al.*, 2006). The disease may be complicated to severe dehydration because lack of electrolytes and fluids that lead to imbalance of electrolytes and then shock or may be cause death (Mohammad *et al.*, 2008).

Defense of immune system is vital for destruction of the *G. lamblia* during the period of infection and progress of effective immunity against it (Baqai *et al.*, 2000). Cytokines has principle role in *G. lamblia* infection. Interleukins are produced by immune cells lymphocytes, macrophages and monocytes. They act on other cells of the immune system to regulate their function. In parasitic diseases the interleukins illicit the inflammatory reactions. Interleukin-4 belongs to family of chemokine and suggested possess a role in contribute of chronic inflammation (Bayraktar *et al.*, 2005).

The current work aimed to investigates the effect of *G. lamblia* infection on some immunological parameters such as, IL-10, IL-4 and immunoglobulin IgA, by ELISA technique in patients with giardiasis and control group.

### Materials and Methods

In the present study, one hundred fecal and blood samples were recovered from patients who admitted to Marjan teaching hospital and Babylon teaching hospital for Maternity and children in Babylon province, Iraq, during the time from June to August 2018. The enrolled patients (aged from 1 year-50 year) were 46 males and 54 females.

### Patients and Samples collection

Stool sample were examined under microscopy by using direct smear for *G.lamblia* detection, Freshly stool samples were processing and analyzed by direct microscopy using X40 power to demonstrate *Giardia lamblia* (trophozoites and cysts) (Ali and Hill, 2003).

Blood samples were assembled from patients and healthy individuals, centrifuged at 3000 rpm for 5 minutes (Backman/counter, Germany) to isolate serum. Each serum sample was divided into three parts; each of them was kept in deep freeze at -20°C till used. Serum interleukins (IL-4, IL-10) were estimated in patients and controls by ELISA test.

### Assessment of serum IL-10 and IL-4 concentrations by ELISA Test

ELISA test used for estimation of human IL-10 according to the manufacturer instructions. US Biological IL-10 kit protocol/ United State Biological, catalog Number 18432-05. Measurement of interleukin-4 concentration was done according to the manufactures instructions by using specific kit.

### Assessment of serum IgA Level by using Single Radial Immunodiffusion (SRID) test

This procedure consists of immune precipitation in agarose between the antigen. and its homologous antibody. It is performed by incorporating one of the two immune reactants (usually antibody) uniformly throughout a layer of agarose gel, and then introducing the other reactants (usually antigen) into wells duly punched in the gel. Antigen-diffuses radially out of the well into the neighboring gel-antibody mixture. An observable ring of precipitation forms. Ring diameters are measured by an ocular. Unknown concentration.is concluded from the tables accompanied with each type of plate (Bienz *et al.*, 2003).

## Statistical Analysis

Statistical analysis of data was carried out using SPSS version 21 (SPSS, IBM Company, Chicago, USA). Categorical variable were presented as frequencies and percentage (Al-Rawi and Khalafalla, 2000).

## Results

The results of this study found that out of 100 stool samples, 34 samples were found to be positive for *G. lamblia*, the infection rate noted 34%. The positive samples comprised of 21 male and 13 female with infection rates recorded as 34.4% and 33.3% respectively. The results statically indicate no difference between them (Table 1).

**Table 1:** Distribution of giardiasis infection depending gender

Gender	Exam No.	%	Infected No.	%
Male	61	61	21	34.4
Female	39	39	13	33.3
Total	100	100	34	34

The results reveal that there was a significant relation between age group and infective rate of *G. lamblia* at ( $P < 0.05$ ). Results also demonstrate a high infection rate (52%) in the age group (1-10) years (Table 2).

**Table 2:** Distribution of giardiasis according to age group

Age	Exam No.	%	Infected No.	%
1-10	46	46	24	52
11-20	11	11	1	9
21-30	7	7	0	0
31-40	6	6	1	16.6
41-50	30	30	8	26.6
Total	100	100	34	34

Our results showed the interleukin levels (IL-4 and IL-10) of infected group was higher than those of controls group (Table 3). The increase ratio of IL-4 and IL-10 was appears statistically significant ( $P < 0.05$ ).

**Table 3:** Comparison between patients and control interleukins concentration

Testing group	No.	Mean pm/ml $\pm$ SE	
		IL-4	IL-10
Infected	34	0.26 $\pm$ 0.49	0.21 $\pm$ 0.10
Uninfected	20	0.14 $\pm$ 0.11	0.18 $\pm$ 0.10

Significant differences at ( $P < 0.05$ ), SE: Stander error

The present results emphasize the mean levels of IgA in patients and control were (538.4, 489.9 mg/dL) respectively (Table 4). The IgA levels were increased significantly ( $P < 0.05$ ) in infected group in compare to control group.

**Table 4:** Mean level of immunoglobulin (IgA) concentration in patients and control

Testing group	Mean mg/dl
Infected group	538.4
Uninfected group	489.9

Significant differences at ( $P < 0.05$ )

## Discussion

The results of this study found that out of 100 stool samples, 34 samples were found to be positive for *G.*

*lamblia*, the infection rate noted 34%. The positive samples comprised of 21 male (34.4%) and 13 female (33.3%), the results showed no significant difference between infection rates of them (Table 1).

Giardiasis is a disease that found in all world, but spread more in the third world countries like Iraq, where is poor sanitary and living conditions (Al-Saeed and Issa, 2006).

This finding may be contingent on some factors like accessibility to clean drinking water, perfect environmental conditions, and low level of individual hygiene (Ulukanligil and Seyrek, 2004).

The prevalence rate was slightly higher in males than females. According to sex the result showed that no differences in the infection rate. Other similar results confirm that distribution of 52.8% male and 47.2% female (Cláudia *et al.*, 2012). This work was also compatible with other study which confirmed that the infection rate was 3.19%. The giardiasis rate of female was 1.7% while it was 5.26% for male patients (Mohammad *et al.*, 2008). (Kim *et al.*, 1997) studied the *G. lamblia* infection in Korean population and they found that the occurrence of *G. lamblia* by sex were (1.28 %) for female and 3.84 % for male patients. The current results were also compatible with Al-Saeed and Issa form Kurdistan region in Iraq (Al-Saeed and Issa, 2006).

The results appears that there was a significant correlation between Age group and infection rate of *G. lamblia* at ( $P < 0.05$ ). Results also indicate that the infection is high percentage in the age group (1-10) years (Table 2).

The high occurrence rate in this study may be due to the lack of effective immunity against different pathogenic microorganism as this age group is relatively less resistant to this infection as reported by several authors worldwide (Farthing, 1993). A factors such as poor health hygiene, low socioeconomic status and environment conditions (Faubert, 2000). In addition, children are more contact with land and its contamination, sharing things among themselves in addition level of mother's education increase the risk of infection, all these factors may be responsible for increase percentage of infection in this age. In agreement to this study, (Minvielle *et al.*, 2004) reported higher prevalence (11%) of giardiasis in children with this age group.

Our results showed the interleukin levels (IL-4 and IL-10) of infected group was higher than those of controls group (Table 3). The increase ratio of IL-4 and IL-10 was statistically significant ( $P < 0.05$ ). The results of the present study was in accordance with several authors worldwide (Bayraktar, 2005; Mahmud *et al.*, 2018). However, this finding was incompatible with the results obtained by (Mitra *et al.*, 2012) who approved that the level of interlukin 4 was decreased in patients with Giardiasis.

The specific interleukin produced by Th2 lymphocytes like as (IL-5, IL-4, IL-10), major interleukine responsible for the accumulate the eosinophil population in enteric protozoal infection is interleukin-4 and has an essential role in the eradication of parasitic illness (De-Waals and Moree, 1988). In *G. lamblia* infection, most of the cytokines are made by CD<sup>4+</sup> of Peyer's patches or created from the mucosa of the lymphoid tissue (MALT) owing to long period of antigenic stimulation via the parasite (Beutler and Cerami, 1989). The nature and the quantity of these cytokine responses may be

disturbed by the infecting parasite and then cause damage to mucosa lining the small intestine.

The results revealed significant increase in level of IL-10 which may state the function of T-helper 2 interleukins to activate immune response against parasitic infection. Infection with this parasite mainly causes diarrhea and malabsorption and the T-cell activation, and interleukins release related to mucosal inflammatory produced. Several authors such as (Cotton *et al.*, 2014) revealed that intestinal lining epithelial interleukins and other interleukins may have effect in mechanism defense of immunity to giardiasis.

The IgA levels were increased significantly ( $P < 0.05$ ) in infected group in compare to control group. The results are concur with study done by (AL-Masoudi *et al.*, 2015). who indicate to elevated level of IgA in patients group compared to control group. giardiasis may stimulate the production of this immunoglobulin In humans, the infection with *Giardia* parasite causes increase in Immunoglobulin G and Immunoglobulin A. As IgA is implicated in the removal of *G. lamblia* from the gastrointestinal tract, it gives the impression to have a vital role in the immune response (Walterspiel *et al.*, 1994). The occurrence of specific anti *Giardia* sIg A was established in duodenum of infected individuals (Eckmann, 2003).

### Conclusion

Our results showed that infective rate of *G. lamblia* was 34%. High infection was noted in males rather than females. Also, interleukins 4 and interleukins 10 were high in infected group than those of controls group. On the other hand, the significant elevation in IgA levels in infected group in compare to control group.

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